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Evaluation of the impact of dissolved oxygen concentration on biofilm microbial community in sequencing batch biofilm reactor

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The effect of dissolved oxygen concentration (DO) during simultaneous nitrification and denitrification (SND) was investigated in a sequencing batch biofilm reactor (SBBR). In addition, the removal rates of nitrogen and bacterial communities were investigated under different concentrations of DO (1.5, 3.5, and 4.5 mg/L). When the SND rate was 95.22%, the chemical oxygen demand and nitrogen removal was 92.22% and 84.15%, respectively, at 2.5 mg/L DO. The denitrification was inhibited by the increase of oxygen concentration. Microelectrode measurements showed that the thickness of oxygen penetration increased from 1.0 mm to 2.7 mm when the DO concentration increased from 1.5 mg/L to 5.5 mg/L. The current location of the aerobic and anaerobic layers in the biofilm was determined for analysis of the microbial community. High-throughput sequencing analysis revealed the communities of the biofilm approached similar structure and composition. *Uliginosibacterium* species, biofilm-forming bacteria *Zoogloea* species and *Acinetobacter* species were dominant. In the aerobic layer, phyla *Betaproteobacteria* and *Saprospirae* were predominant, the major phyla were shifted from *Proteobacteria* followed by *Firmicutes* and *Bacteroidetes*, which comprised 82% of the total sequences during the SND period. *Anaerolineae* was dominated in the anaerobic layer. The high abundance of *Nitrospira* in the aerobic biofilm provides evidence of the SND system performing better at ammonia oxidization. In addition, real-time PCR indicated that the amount of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) matched the *Nitrospirales* and *Nitrosomonadales* abundance well. Collectively, this study demonstrated the dynamics of key bacterial communities in the SND system were highly influenced by the DO concentration.

[**Key words:** Simultaneous nitrification and denitrification; Dissolved oxygen concentration; Microbial community; Real-time polymerase chain reaction; SBBR]

When compared with the activated sludge process, biofilm technology has a smaller layout area, greater microbial population, stronger impact resistance, and more stable ecological system (1,2). In addition, biofilms have higher efficiency and wider application in nitrogen removal systems than activated sludge (3,4). Ammonia-oxidizing bacteria activities are typically observed in the outer aerobic zone of both biofilm and aggregates, while anammox bacteria are predominant in the inner anoxic zone, where they are protected from oxygen inhibition. Therefore, an ideal reactor configuration should facilitate oxygen transfer and have reliable biomass retention (e.g., biofilm or granular-based processes).

In biological nitrogen removal, traditional nitrification and denitrification follow two principal steps, ammonia oxidation and the conversion of oxidation products (and ammonia) to nitrogen gas, which can be simultaneously achieved in the aerobic and anaerobic layers inside the biofilm (5,6). Simultaneous nitrification and denitrification (SND) implies that these processes take place concurrently in the same reaction zone under the same overall operating conditions. Layered and orderly ammonia oxidation and nitrogen gas generation are crucial to SND.

In biofilm technology, the transfer and consumption of dissolved oxygen (DO) plays important roles in nitrogen removal. Excessively high DO transfer resistance in the biofilm makes the aerobic layer too thin and complicates ammonia oxidation. Conversely, excessively low DO transfer resistance slows the rate of denitrification. However, nitrification is carried out by two different groups of organisms, the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB), respectively. Because of the high sensitivity of AOB to many environmental factors, they are primarily responsible for the first and often rate-limiting step in nitrification (7). Dissolved oxygen is usually considered the most important factor impacting AOB numbers during SND. Therefore, knowing how to control DO concentration and transport to achieve nitrification in the aerobic layers and denitrification in the anaerobic layers of biofilm is essential to SND. It is also important to determine the AOB quantity and DO concentration of the aerobic and anaerobic layers during SND individually. However, a convenient and fast method for determining the nitrifying bacteria community structure of the aerobic and anaerobic layers is lacking.

The object of this study was to quantify microenvironment variation of the SBBR biofilm at different DO concentrations. Microelectrodes have been considered a powerful tool for profiling the biofilm microenvironment (8). The transport of DO and nutrients from bulk solution to the inner part of the biofilm is more closely related to the thickness of the aerobic and anaerobic layers

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than to the biofilm structure (9). Hence, the microelectrode was used to determine the current location of the aerobic and anaerobic layers in the biofilm. Real-time PCR was used to analyze and compare the quantity of nitrifying bacteria at three locations in biofilm at each DO concentration. Biofilm community compositions were examined by high-throughput sequencing, while multivariate statistical tools were used to further elucidate the potential impact and contribution of operational parameters to the community structure and abundance. Samples were collected from the aerobic layer, the mass transfer boundary layer, and the anaerobic layer according to the results of the microelectrode analysis.

MATERIALS AND METHODS

Samples of biofilm and description of SBBR treatment systems A schematic representation of the SBBR is given in Fig. 1. The reactor had a working volume of 20 L. Biofilms of the same construction were placed into the reactor for the entire process. A submerged pump was placed on the bottom of the reactor and used to circulate the synthetic water so that sludge in the reactor moved. Air was introduced through a distributor on the bottom of the reactor from an air compressor. Sludge (with 59.6% NH_4^+–N removal efficiency) collected from the secondary sedimentation tank in a local municipal wastewater treatment plant was seeded in the reactor. The reactor was fed sequentially with synthetic wastewater containing the materials shown in Table 1. The synthetic feed water was supplied with $C_6H_{10}O_5$ (266 mg/L), NH_4Cl (85 mg/L), KH_2PO_4 (25 mg/L) and other mineral salts as follows: $MgSO_4$ (33 mg/L); $CaCl_2$ (20 mg/L); $FeSO_4 \cdot 7H_2O$ (4 mg/L). The hydraulic retention time (HRT) was set at 12 h; instantaneous FILL, 0 h; REACT, 10 h; IDLE, 2 h; instantaneous DRAW, 0 h. The experiments were conducted under the following standard conditions: 30 ± 2 °C, pH of 7.5 ± 0.3 and DO of 3–4 mg/L. The pH inside the reactor was maintained between 7 and 7.5 by automatically adding 0.2 M NaOH solution. To maintain a suitable temperature (30 ± 2 °C), warm water from a heating circulator was recirculated into the integrated water jacket (model 1104, VWR Scientific, West Chester, PA, USA).

The reactor was operated for six days without sludge discharge so that a biofilm was cultured. Activated sludge discharged after the surface of biofilm covering a thin and cream colored biomembrane. The reactor was completely started up when the removal efficiency of COD, ammonium and TIN achieved 90%, 90% and 70%, respectively. At this stage, the reactor had achieved SND.

While the reactor had kept stable, the influent ammonia nitrogen concentration was 27–30 mg/L, and the aeration was adjusted to obtain different dissolved oxygen concentrations in three phases. At the first phase (until 24 days), the aeration was adjusted to 200 mL/min, corresponding to a DO of 1.3–1.8 mg/L. At phase II (from day 25–54), the DO in the SBBR was controlled at about 3.5 mg/L by adjusting the aeration. Moreover, DO in the reactor increased further to be higher than 4.5 mg/L at phase III.

TABLE 1. Composition of synthetic wastewater.

	COD	TIN	NH_4^+–N	NO_3^-–N	NO_2^-–N	pH
Concentration (mg/L)	295–312	27.4–30.9	27.4–30.0	0	0–0.9	7.3–7.8

The biofilm was cultured for 30 days, after which it was carefully removed from the SBBR for biofilm microelectrode measurement. The samples collected from the biofilm were transferred to a 1.5 mL Eppendorf tube and kept for extraction of genomic DNA. Three samples were then collected in one biofilm according to the microelectrode results with the goal of collecting samples from the aerobic layer, mid-aerobic layer and anaerobic layer. However, the mid-aerobic layer was the dividing line between the aerobic and anaerobic layer.

Polymeric fibrous carriers Polymeric fibrous carriers (PFC) were obtained from polyamide (PA), polypropylene (PP) and polyethylene (PE) by melt-blowing with fiber diameter 150 mm, density 8–9 kg/m^3, and specific surface area 1236 m^2/m^3. The carriers are shown in Fig. 1. The PFC combined the characteristics of the soft carriers and the semi-soft carriers. Its excellent performance such as large specific surface area and strong adhesion, can protect the biofilm from fluid shearing and collision and accumulated enough microorganisms in the carrier. However, the filaments were evenly distributed around the circular skeleton. This results in stronger gas cut and improved the utilization rate of oxygen, providing ideal conditions for SND.

Water quality analysis Water quality analysis was conducted according to the following standard methods (58). Concentrations of ammonium (NH_4^+–N), nitrite (NO_2^-–N) and nitrate (NO_3^-–N) were measured daily in the influent and effluent by colorimetric methods using a spectrometer (UV2550, Shimadzu, Kyoto, Japan). Total inorganic nitrogen (TIN) was calculated from the sum of NH_4^+–N, NO_2^-–N and NO_3^-–N as NH_4Cl was the only nitrogen source in the synthetic wastewater. The COD was determined by the oxidation method using potassium dichromate. The pH and dissolved oxygen were measured directly with electrodes housed in the reactor (HQ40d, Hach, Loveland, CO, USA).

Microelectrode measurements The DO microelectrode automation system (PA2000, Unisense, Denmark) consisted of three parts: a computer program, a motion control system, and a data acquisition system. All components and connections are shown in Fig. 2. The acquisition system is combined with a microelectrode (OX-N, Unisense), picoammeter (OXY meter, Unisense), and a computer program (Sensortrace PRO V3.1.3, Unisense). The acquisition system was used to collect, compute and store the data gathered from the oxygen microelectrode, which was the most important part of the automation system. The diameter of the tip of the combined oxygen microelectrode was 1 mm. The

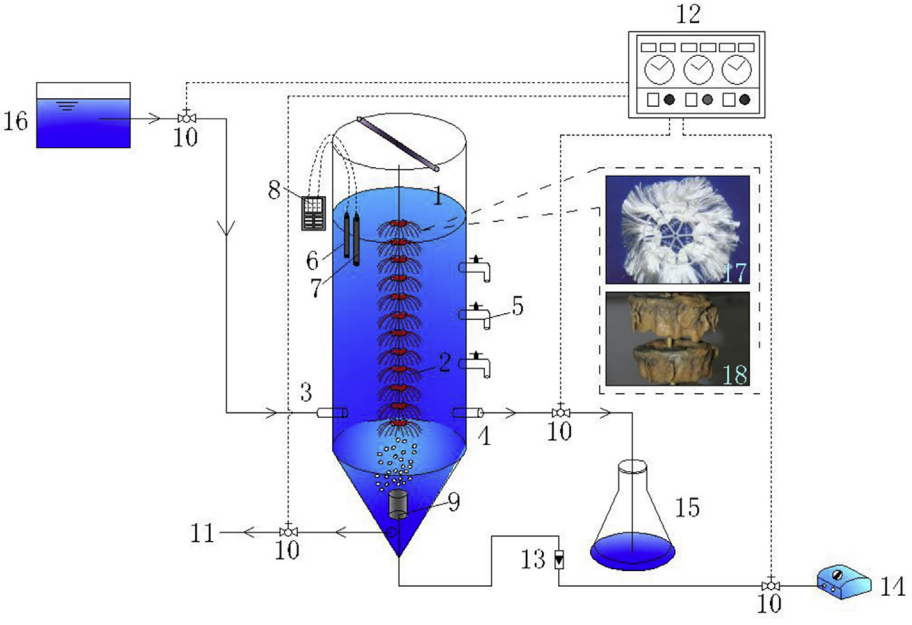

FIG. 1. Schematic representation of the SBBR and the photograph of polymeric fibrous carriers (1, SBBR reaction; 2, biofilm; 3, influent pipe; 4, effluent pipe; 5, sample outlet; 6, dissolved oxygen electrode; 7, pH electrode; 8, DO and pH meter; 9, aerator; 10, solenoid valve; 11, excess sludge; 12, time control; 13, air flow meter; 14, air compressor; 15, treated effluent; 16, feeding tank; 17, image of polymeric fibrous carrier; 18, image of biofilm in carrier).

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